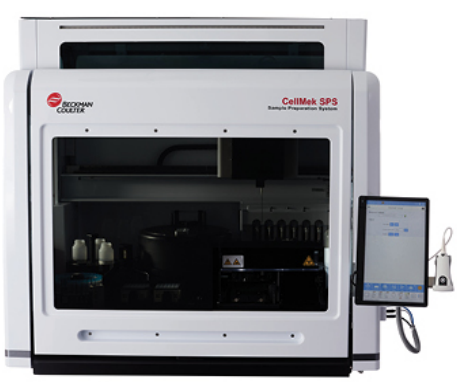

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CTLA-4 Exon 1 Polymorphism in Patients With Autoimmune Blood Disorders

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CTLA-4 is a CD28 homologue that plays an important role in negative regulation of T-cell responses. Its transient expression on the surface of activated T cells antagonizes the activating signals and terminates the T-cell response. An A to G polymorphism at position 49 of the CTLA-4 first exon has recently been associated with several autoimmune disorders. In the present study we have examined the prevalence of the A and G alleles of the CTLA-4 gene in 50 patients with autoimmune hemolytic anemia (AIHA), of which 20 had idiopathic AIHA and 30 had AIHA and chronic lymphocytic leukemia (CLL), and in 60 patients with immune thrombocytopenic purpura (ITP). Control subjects were 100 healthy individuals and 100 CLL patients without clinical evidence for an autoimmune disease. The G allele was present at a significantly higher frequency among the patients with AIHA ($P = 0.003$), whereas no difference was observed between patients with ITP and controls. The G allele frequency was highest among CLL patients who had developed AIHA. The obtained data indicate that the G allele of CTLA-4 predisposes to the development of AIHA, particularly among patients with CLL. Am. J. Hematol. 72:147–149, 2003.

Key words: autoimmune hemolytic anemia; immune thrombocytopenic purpura; chronic lymphocytic leukemia; CTLA-4 polymorphism

INTRODUCTION

The CTLA-4 molecule is important in the downregulation of T-cell antigen responses. It appears on the surface of T cells following their activation by TCR/peptide/MHC and CD28/B7 interactions and inhibits further T-cell proliferation by reducing CD28/B7 interactions because of its higher affinity for B7. In addition, CTLA-4 engagement can mediate downregulation of T-cell responses in the absence of CD28, indicating that CTLA-4 may have independent inhibiting properties [1].

A number of recent studies have implicated CTLA-4 in the pathogenesis of autoimmune diseases. CTLA-4-deficient mice show a severe lymphoproliferative disorder with massive autoimmune tissue destruction [2]. CTLA-4 is also deficiently expressed in non-obese diabetic mice, which is an animal model of autoimmune diabetes [3]. Furthermore, an A to G polymorphism at position 49 of the CTLA-4 first exon has been associated with the development of insulin-dependent diabetes mellitus in several ethnic groups [4]. This polymorphism results in an amino acid substitution (Thr-Ala) at codon 17 of the CTLA-4 leader peptide. A high prevalence of the CTLA-4 G allele, which apparently confers susceptibility to autoimmune disease, has also been reported in patients with Graves' disease, Hashimoto's thyroiditis, rheumatoid arthritis, multiple sclerosis, primary biliary cirrhosis, and in some studies of patients with systemic lupus erythematosus [5–9].

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TABLE I. Distribution of the A/A, G/A, and G/G Genotypes in Patients and Controls

	A/A	G/A	G/G	Total
ITP	26 (43%)	27 (45%)	7 (12%)	60
Primary AIHA	9 (45%)	11 (55%)	0 (0%)	20
CLL + AIHA	8 (26.6%)	20 (66.6%)	2 (6.6%)	30
CLL without AIHA	53 (53%)	33 (33%)	14 (14%)	100
Normal controls	51 (51%)	39 (39%)	10 (10%)	100

In this study we have investigated the possible contribution of the CTLA-4 G allele in the pathogenesis of autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura. We find a significant overrepresentation of this allele among patients with AIHA, further implicating the CTLA-4 G allele as a susceptibility factor for autoimmune diseases.

PATIENTS AND METHODS

The study group consisted of 50 patients with autoimmune hemolytic anemia (20 patients with idiopathic AIHA and 30 patients with CLL and secondary AIHA) and 60 patients with immune thrombocytopenic purpura. Diagnosis of AIHA and ITP were established on the bases of standard clinical and laboratory parameters. Controls were 100 healthy individuals and 100 CLL patients without clinical and laboratory evidence for an autoimmune disease during the period of follow-up. Samples from the patients with CLL and AIHA were collected over a longer period of time (1995–2002), which is the reason for the high number of such patients in our series. Informed written consent for genetic studies was obtained from all patients and controls.

DNA was isolated from peripheral blood leukocytes by phenol/chloroform extraction. Amplification of the target DNA sequence in exon 1 was performed with PCR primers 5′-GTCAAGGGACCATTAGAAG-3′ and 5′-TCATCCCTGTCTTCTGCAA-3′. The amplified fragment was 685 bp in length. The amplified sequences were digested overnight at 37°C with 0.5 U of the *Bbv*I restriction enzyme (New England BioLabs, Beverly, MA). Digested fragments were analysed on 3% agarose gels.

Distribution of alleles in patients and controls were compared by the χ^2 test using Microsoft Excel software (Microsoft Corporation, Redmond, WA). $P < 0.05$ was considered significant.

RESULTS

The distribution of the CTLA-4 genotypes among the different subgroups is shown in Table I. The G allele was significantly more prevalent among the patients with AIHA compared to the controls, which included healthy individuals and CLL patients without clinical and laboratory evidence for AIHA during the period of follow-up ($P = 0.003$). We also compared the prevalence of the CTLA-4 genotypes between the two groups of patients with CLL, because of the frequent association of AIHA with this disease. Among the 130 cases investigated, 30 had a prior episode of AIHA. Again, a strong correlation was observed between the presence of the CTLA-4 G allele and the development of AIHA in this uniform group of patients ($P = 0.004$).

There was no difference in the presence of the CTLA-4 G allele between patients with ITP and controls ($P = 0.43$).

DISCUSSION

Autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura are the two most common autoimmune blood disorders. Both may occur as primary disorders or secondary to other lymphoproliferative or immune diseases. Their pathogenesis appears to be multifactorial, involving disturbances in regulatory and autoactive B- and T-cell subsets. In the present study, we investigated the possible contribution to this dysregulation of a polymorphic variant of the immunoregulatory molecule CTLA-4. The presence of the CTLA-4 G allele, which has been associated with several autoimmune disorders, correlated strongly with the development of AIHA but not with the development of ITP. This correlation was particularly evident in the group of patients with CLL, which included a large series of patients with secondary AIHA. The G allele was present in 73% of the CLL patients that had developed AIHA compared to 47% of the control CLLs. For unknown reasons, CLL patients frequently develop anti-RBC auto-antibodies, and the presence of the CTLA-4 G allele could eventually magnify this autoimmuune response. Recently, the CTLA-4 G allele was shown to have a less potent inhibitory effect on T-cell proliferation in vitro and was associated with reduced expression of cell-surface CTLA-4 molecules upon T-cell activation [9,10].

In summary, our results indicate that CTLA-4 may play a role in the pathogenesis of autoimmune hemolytic anemia, particularly among patients with CLL. The lack of association with ITP is intriguing, but it may indicate that features other than the extent of the antibody response are more important in the pathogenesis of this disorder.

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